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Phytates: Occurrence, Bioavailability and Implications in Poultry Nutrition

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1 INTRODUCTION

Phytates. [Myoinositol 1,2,3,4,5,6-hexakis (dihydrogen phosphate)] are found in all plants where they are generally regarded as the chief storage form of phosphorus in seeds and vegetative storage tissues. Phytates rapidly accumulate in seeds during the ripening period,^{1,2,3} accompanied by other storage substances such as starch and lipids. In mature seeds, phosphorus is present primarily in the form of phytic acid as a complex salt of calcium, magnesium and potassium, and/or with proteins.⁴ Phytic acid serves several important physiological functions during dormancy and germination of seeds. These include initiation of dormancy,⁵ antioxidant protection during dormancy⁶ and storage of phosphorus, high-energy phosphoryl groups and cations for use during germination.⁷

Since a major portion of poultry diets consists of plant-derived ingredients, phosphorus from the phytate assumes considerable nutritional significance. In addition, a heightened environmental awareness has created a renewed interest in phytates in recent years.⁸ Under normal dietary conditions, phytate phosphorus is either unavailable to, or poorly utilized by, poultry⁹ due to insufficient quantities of endogenous avian phytase. This inability, or inadequacy, of poultry to utilize phytate phosphorus presents two problems to the producers.¹⁰ Firstly, it necessitates the addition of inorganic phosphorus sources to the diets; of the mineral supplements added to feed formulations, inorganic phosphorus sources are the most expensive. Secondly it results in the excretion of large amounts of phosphorus in the manure, posing an environmental concern especially in areas of intensive animal production. Also the ability of phytate to complex with metal ions is well known and is another nutritional concern associated with phytate.

Excellent summaries of the early research on the role of phytate in animal nutrition^{11,12} and poultry nutrition^{9,13} have been presented. Several reviews on

phytates have also appeared in relation to their chemistry,^{14,15} methods of analysis^{16,17} and, significance in cereal technology¹⁸ and human nutrition.^{19,20} The current review will focus on phytate research relevant to poultry and examine the nutritional implications of recent developments for poultry production.

2 OCCURRENCE OF PHYTATES IN FEEDSTUFFS

Phytate phosphorus constitutes the major portion of total phosphorus in seeds of cereals, grain legumes and oil-bearing plants. In general, the proportion of phytate phosphorus varies from 60 to 80% of the total phosphorus in these materials (Table 1).

The concentration of phytate phosphorus in feedstuffs depends largely on the part of the plant from which they are derived. Oilseed meals and cereal by-products contain large amounts of phytate phosphorus, whereas cereals and grain legumes contain moderate amounts (Table 1). Roots and tubers contain low amounts of phytate phosphorus. In vegetative, non-storage organs of plants, such as the leaves, phytate phosphorus is either absent or present only in trace amounts. Among the common feedstuffs, sesame meal and rice bran have the highest levels of phytate.

The phytic acid in cereals is not uniformly distributed within the kernel, but associated with specific morphological components in the seed.³² In wheat and rice, the endosperm is almost devoid of phytate, but the aleurone layers of the kernel and the bran contain substantial amounts. In rice, more than 80% of the phytate is present in the outer bran.^{21,33} Corn differs from other cereals since almost 90% of phytic acid is concentrated in the germ portion of the kernel.^{33,34} In dicotyledonous seeds, including oilseeds and grain legumes, the phytate is distributed throughout the kernel in subcellular inclusions, known as globoids,¹⁵ that are found within protein bodies. In contrast, phytates in

Table 1. Phytate phosphorus contents of feed ingredients.

Ingredient	Phytate phosphorus, g/100g DM	Phytate P, as % of total P	Reference
Cereals			
Corn (<i>Zea mays</i>)	0.24 (0.17-0.29) ^a	72 (66-85) ^a	13,21,23,24,27,29
Barley (<i>Hordeum vulgare</i>)	0.27 (0.19-0.33)	64 (56-70)	13,23,25,26
Wheat(<i>Triticum aestivum</i>)	0.27 (0.17-0.38)	69 (60-80)	13,23,24,26,27
Oats (<i>Avena sativa</i>)	0.29 (0.22-0.35)	67 (59-78)	13,26,27
Sorghum (<i>Sorghum vulgare</i>)	0.24 (0.21-0.28)	66 (64-69)	27,29
Foxtail millet(<i>Setaria italica</i>)	0.19	70	29
Finger millet(<i>Eleusine coracana</i>)	0.14	58	29
Rice(<i>Oryza sativa</i>),unpolished	0.27 (0.25-0.28)	77 (74-81)	21,29
Rice, polished	0.09 (0.04-0.17)	51 (49-55)	29,30,31
Cereal by-products			
Rice bran	10.31 (10.02-10.09)	80 (72-86)	24,27,29
Wheat bran	0.92 (0.88-0.96)	71 (70-72)	24,27
Rice polishings	20.42	89	27
Roots and tubers			
Cassava(<i>Manihot esculenta</i>) root meal	04 (0.03-0.04)	28 (25-33)	13,23,29
Sweet potato(<i>Ipomea batatas</i>) tuber meal	0.05	24	29
Taro(<i>Colocasia esculenta</i>) corm meal	0.09	24	29
Grain legumes			
Field peas (<i>Pisum sativum</i>)	0.24	50	23
Cowpeas (<i>Vigna unguiculata</i>)	0.26 (0.22-0.28)	79 (72-86)	25,29
Green gram (<i>Vigna radiata</i>)	0.22 (0.19-0.24)	63 (58-67)	25,29
Pigeon peas (<i>Cajanus cajan</i>)	0.24	75	29
Chickpeas (<i>Cicer arietinum</i>)	0.21	51	29
Oilseed meals			
Soybean (<i>Glycine max</i>) meal	0.39 (0.37-0.42)	60 (57-61)	23,24,26,29
Cottonseed (<i>Gossypium spp0.</i>) meal	0.84 (0.75-0.90)	70 (70-71)	27
Peanut (<i>Arachis hypogaea</i>) meal	0.48	80	22
Rapeseed (<i>Brassica napus</i>) meal	0.70 (0.54-0.78)	59 (43-70)	27,28
Sunflower (<i>Helianthus annus</i>) meal	0.89	77	13
Coconut (<i>Cocos nucifera</i>) meal	0.29 (0.26-0.33)	49 (43-56)	23,29
Sesame (<i>Sesamum indicum</i>) meal	10.18 (10.03-10.46)	81 (77-84)	21,27,29
Miscellaneous			
Grass meal	0.01	2	13
Alfalfa (<i>Medicago sativa</i>) meal	0.02 (0.01-0.03)	12 (5-20)	13,27
Ipil-Ipil(<i>Leucaena leucocephala</i>) leaf meal	0.02	9	29
Cassava leaf meal	0.04	10	29
Corn gluten meal	0.41 (0.29-0.63)	59 (46-65)	23,24,27
Isolated soy protein	0.48	60	27

soyabean are unique in that, although associated with globoids, they appear to have no specific site of localization.

Phytate concentrations in plant materials may vary depending on the stage of maturity, degree of processing, cultivar, climatic factors, water availability, soil factors, location and the year during which they are grown.²⁰ The significant cultivar effect on the phytate phosphorus contents of wheat, soybeans, beans^{26,35} and triticale³⁶ is well documented. Asada *et al.*² reported that phytate phosphorus in rice and wheat increased with maturity of grain and with phosphate fertilization. Bassiri and Nahapetian³⁷ found that wheat cultivars grown under dry climatic conditions had lower concentrations of phytic acid than those grown with irrigation. Significant location and year effects have also been reported for the phytate contents of wheat, rye, triticale and oats.^{36,38,39,40}

3 BIOAVAILABILITY OF PHYTATE PHOSPHORUS FOR POULTRY

Agreement on the degree of utilization of phytate phosphorus by poultry is not apparent in the literature despite almost half a century of research. Most early research indicated that natural phytate phosphorus was poorly utilized by poultry.^{9,12,13} However, it is now becoming increasingly clear that phytate phosphorus is digested and utilized, to some extent, by poultry.

Available literature indicates that phytate phosphorus utilization by poultry ranges from zero^{41,42} to over 50%.^{43,44,45} In balance studies, Nelson⁴¹ determined the hydrolysis of phytate phosphorus from different diets by 4- and 9-week-old broiler chickens and by laying hens. On corn-based diets, the amounts hydrolysed were 0, 3 and 8%, respectively. The corresponding values on wheat-corn based diets were 8, 13 and 13%, respectively. In contrast, Temperton and Cassidy^{46,47} observed from balance studies that the chick could absorb and retain a large portion of ingested phytate phosphorus. Up to 60% of the phytate phosphorus was retained in the chick's body. Retention was higher when sub-optimal levels of non-phytate phosphorus were fed. Edwards⁴³ also reported high phytate phosphorus retention values (37 - 56%) for chicks. In the studies of Ballam *et al.*⁴⁸, phytate hydrolysis values ranging from 3 to 42% were determined depending on the level of dietary calcium and the source of fiber.

Thus, wide disagreement exists among researchers concerning the ability of poultry to utilize phytate phosphorus and, as a result, it is not possible to make general statements about its bioavailability to poultry. The disagreement appears to be due, in part, to the complex nature of factors influencing phytate hydrolysis

including the source of phytate, age of birds, and dietary levels of calcium and vitamin D₃. Part of the variability also arises from differences in the method of phytate analysis and experimental techniques. There also appears to be some confusion as to what constitutes 'bioavailability' or 'utilization' of phytate phosphorus. The criteria of response to define its bioavailability have been many and varied, and include percent apparent digestibility, percent apparent absorption, percent retention, percent apparent availability and others. In most studies, the disappearance of phytate during the passage of feed through the total tract was measured as the indicator of phytate phosphorus utilization. Such disappearance, however, does not mean that the released phosphorus was utilized by the bird, especially if the phytate is hydrolysed by the microbes in the hindgut where phosphorus absorption is negligible.

Several studies have evaluated the utilization of isolated phytate in various chemical forms: phytic acid, sodium phytate and calcium phytate. In a series of experiments, Harms *et al.*⁴⁹ reported that phosphorus from phytic acid was available for growth and bone calcification, and the availability was equal to that of dicalcium phosphate. Waldroup *et al.*,^{50,51} comparing phosphorus bioavailability of dicalcium and monosodium phosphates to those of sodium phytate, calcium phytate and chemically isolated phytic acid, similarly found that phosphorus from phytic acid was as available to the chick as those from inorganic phosphate supplements. Phosphorus in sodium phytate was less available than that in phytic acid while the phosphorus in calcium phytate was essentially unavailable. In a subsequent study, Waldroup *et al.*⁵² determined the availability of phosphorus from organic phytic acid without chemical isolation from the plant material and found that organic phytate phosphorus was equal or superior to inorganic phosphate supplements for chick growth, but somewhat less well utilized for bone calcification. Miller,⁵³ using a plant material-based diet, also showed that phytate phosphorus was able to supply the phosphorus requirements of Coturnix quail.

4 PHYTATE HYDROLYSIS IN POULTRY

In order for phosphorus to be utilized by poultry, phytate must be hydrolyzed into inorganic phosphorus within the digestive tract. Although non-enzymatic cleavage of phytates has been suggested by some workers,^{54,55} the dephosphorylation of phytic acid is largely the result of phytase (myo-inositol hexaphosphate phosphohydrolase) activity. Phytases comprise a family of enzymes that catalyze the stepwise removal of inorganic

Table 2. Phytase activity of some feed ingredients^a.

Ingredient	Phytase activity, units/kg ^b
Cereals and by-products	
Wheat	1193 (915-1581) ^c
Barley	582 (408-882)
Rye	5130 (4132-6127)
Corn	15 (0-46)
Sorghum	24 (0-76)
Wheat bran	2957 (1180-5208)
Rice bran	122 (108-135)
Roots and tubers	
Cassava roots	6 (0-40)
Sweet potat tubers	26 (0-73)
Grain legumes	
Field peas	116 (36-183)
Lupins	0
Oilseed meals	
Soybean meal, 48%	8 (0-20)
Peanut meal	3 (0-8)
Rapeseed meal	16 (0-36)
Miscellaneous	
Alfalfa meal, dehydrated	60 (15-250)
Corn distillers grain	385 (141-850)
Soybean hulls	99 (0-150)

^a Data from Eeckhout and De Paepe.⁷³

^b One unit is defined as the amount of phytase which liberates inorganic phosphorus from a 0.0015M sodium phytate solution at a rate of 1 $\mu\text{mol/min}$ at pH 5.5 and 37°C.

^c Values within parantheses refer to range of values determined.

orthophosphate from phytic acid, via inositol pentaphosphate to monophosphates as intermediary products.⁵⁶ This lysis can occur in the digestive tract or in the feedstuff prior to consumption by the bird, provided conditions are conducive for enzymatic action. Two phytases are internationally recognised⁵⁷: a 3-phytase (EC 2.1.3.8) which first hydrolyzes the ester bond at position 3 of *myo*-inositol, and a 6-phytase (EC 3.1.3.26) which commences the dephosphorylation of phytate at position 6. Both eventually fully dephosphorylate the phytate.¹⁸ Generally, in cereals and fungi, 6-phytase is present.

The degradation of phytate in the digestive tract of poultry may be attributed to the action of phytases from one or more of three possible sources. These sources include, (1) intestinal phytase in digestive secretions, (2)

phytase activity originating from microbes resident in the intestinal tract or (3) endogenous phytase activity present in some feedstuffs.

A controversy exists concerning the presence of intestinal phytase activity in poultry. Bitar and Reinhold⁵⁸ claimed that inçivity is found in the mucosa of the small intestine in poultry. Davies and Motzok⁵⁹ also reported that a phytase, obtained from homogenates of the chick intestinal mucosa, hydrolysed sodium phytate. The presence of intestinal phytase in poultry however, has not been confirmed in other studies^{60,61,62}. Whether phytase or other phosphatases of the intestinal mucosa play a role in phytate hydrolysis is yet to be clearly established.^{60,61} Since phytic acid may also act as a substrate for alkaline phosphatases (EC 3.1.3.1) and acid phosphatases (EC 3.1.3.2), it has been suggested that intestinal phytase activity may be a manifestation of such non-specific phosphatases.^{59,62} The finding of Matterson⁶³ that an increase in intestinal phytase activity is accompanied by a decrease of blood alkaline phosphatase lends some support to this suggestion. However, Cooper and Gowing⁶⁴ found that intestinal phytase activity in rats was somewhat similar to but distinct from alkaline phosphatase. Thus the available data on the presence of phytase activity in intestinal secretions of poultry is inconclusive and these conflicting data suggest that endogenous phytase activity in the intestinal mucosa of poultry is extremely low, at least in the younger birds.

Phytase is known to be produced by fungi, bacteria, yeast and by rumen and soil microorganisms.⁵⁶ Filamentous fungi, particularly those from the *Aspergillus* genus (*Aspergillus ficuum* and *A. niger*), have been extensively studied as rich sources of microbial phytase.⁵ The microbes in the rumen of ruminant animals produce phytase which effectively hydrolyses phytate.¹² Nelson *et al.*⁶⁵ reported that total hydrolysis of phytate occurred in the digestive tracts of calves and steers. Thus, it is possible that microbial phytase may be active in the gastrointestinal tract of monogastric animals under certain conditions, although in poultry this appears to be of minor importance in phytate hydrolysis. There is some evidence however, that indicates bacterial phytase may be active in the digestive tract of poultry. Warden and Schaible^{66,67} found that the addition of lysed *E.coli* cellular material, obtained from the intestinal digesta, to a phosphorus-deficient, corn-soybean meal diet improved both growth and calcification in chicks; this response was attributed to phytase or similar enzymes of bacterial origin. If phytase from microbes resident in the digestive tract does hydrolyse the phytate in poultry, then the question arises whether it occurs at a site where phosphorus can be absorbed. As noted earlier, total tract

disappearance of phytate is not indicative of utilization of released phosphorus by the bird.

It has been long established that some feed ingredients have endogenous phytase activity. Phytases are present in most cereals, but their activity varies widely amongst cereals.^{68,69,70} Rye, wheat and barley contain high levels of phytase activity, whereas corn, oats, sorghum and oilseeds contain little or none of the enzyme (Table 2). Milled grains are reported to have more phytase activity than intact grains.⁷¹ High phytase activity was also reported in sun-cured lucerne meal, but was not detected in dehydrated meal.⁷²

In a series of experiments, Temperton and co-workers^{46,47,74,75} provided indirect evidence that endogenous plant phytase is effective in feeds. Using diets containing 32 to 36% wheat and 10% barley meal, without feedstuffs of animal origin and without inorganic phosphate supplements, they demonstrated that the chicks were able to utilize phytate phosphorus for bone calcification. The data of Scheurmann *et al.*⁷⁶ show that wheat phytase can act on phytates from other ingredients as well. Thus it is possible to avoid the use of inorganic phosphate supplements by employing a combination of these ingredients.

It is relevant to note that the optimum pH for plant phytase activity is in the range of 4.0 - 6.0,^{20,77,78} though some activity may be retained at pH 3.0. It is unlikely that significant amounts of the enzyme will survive the highly acidic conditions (pH 1.0-2.5)^{79,80} of the proventriculus. For example, Eeckhout and De Paepe⁷⁷ have shown that wheat phytase is inactive at pH 3.0. However, whether or not this inactivation is reversible when pH increases to 6.0-7.0 in the small intestine^{79,80} is not known. If this is irreversible, then any breakdown of dietary phytate by plant phytases must occur prior to or within the proventriculus before the low pH inactivates the enzyme.

It is however, noteworthy that the effectiveness of wheat phytase in hydrolysing phytate appears variable. For example, Nelson⁴¹ found that laying hens hydrolyzed only 13% of the phytate in wheat-based diets. It is possible that the amount of active phytase in feed ingredients can vary depending on cultivar, age of wheat and/or drying and storage conditions. High temperatures employed during ingredient processing or during pelleting of diets can also influence the native phytase activity of plant ingredients. Plant phytase activity is not altered by such treatments at temperatures between 47° and 62° C,⁷¹ but higher temperatures (70-80° C) can cause partial or total inactivation. Evidence for such inactivation of phytase in wheat, barley and wheat middlings by heating or pelleting has been presented by Jongbloed and Kemme.⁸¹

It should be noted that the activity of phytase in the gastrointestinal tract may be governed by a number of variables including source, pH and the presence of metal ions. Microbial phytases have a broader pH activity range than plant phytases⁷⁷ and therefore are more effective within the gastrointestinal environment. Phytases are known to be activated by several divalent cations, such as calcium, magnesium, zinc and vitamin D. Phytase inhibitors include phytate precipitants such as copper, zinc, iron, calcium, fluoride, inorganic phosphorus and phytate.^{5,18,82}

5 FACTORS INFLUENCING PHYTATE PHOSPHORUS UTILIZATION

The hydrolysis and absorption of phytate phosphorus by monogastric animals are complex processes that are influenced by factors such as: dietary levels of calcium, inorganic (or available) phosphorus and vitamin D₃; age and type of birds; dietary ingredients and feed processing.

5.1 Dietary calcium and phosphorus levels

Phytate phosphorus utilization by poultry has been shown to be influenced by both calcium and phosphorus levels in the diet,^{45,83,84} but the effects of dietary calcium are much greater. At very high levels of calcium, phytate hydrolysis may be completely prevented.¹²

Under practical feeding conditions where calcium and phosphorus must be added to the diets for maximum performance and bone calcification, phytate phosphorus is probably utilized very little, if at all, by poultry.⁸⁵ This adverse influence has been confirmed in several studies where calcium was added at recommended levels.^{41,86} Balance studies by Nott *et al.*⁸⁷ demonstrated that when calcium intake was adequate for optimum shell quality, hens were unable to utilize phytate phosphorus. Ballam *et al.*⁴⁸ found that chicks fed diets containing 1.0% calcium hydrolyzed less phytate than those fed on diets with 0.85% calcium. Similarly, Mohammed *et al.*⁴⁵ reported that phytate phosphorus utilization was increased by 15% when dietary calcium levels were reduced from 1% to 0.5%. In their studies, the adverse effects of a high-phytate, low-inorganic phosphorus diet on chicks were overcome by lowering the calcium level and/or increasing the vitamin D₃ concentration.

Conversely, a rather high availability for phytate phosphorus can be shown under experimental conditions for short time periods with low calcium levels. The influence of low calcium levels on phytate phosphorus utilization by poultry was exemplified by the studies of Nwokolo *et al.*⁸⁸ Using diets containing 0.13 to 0.42% calcium, they obtained high phosphorus availability (70

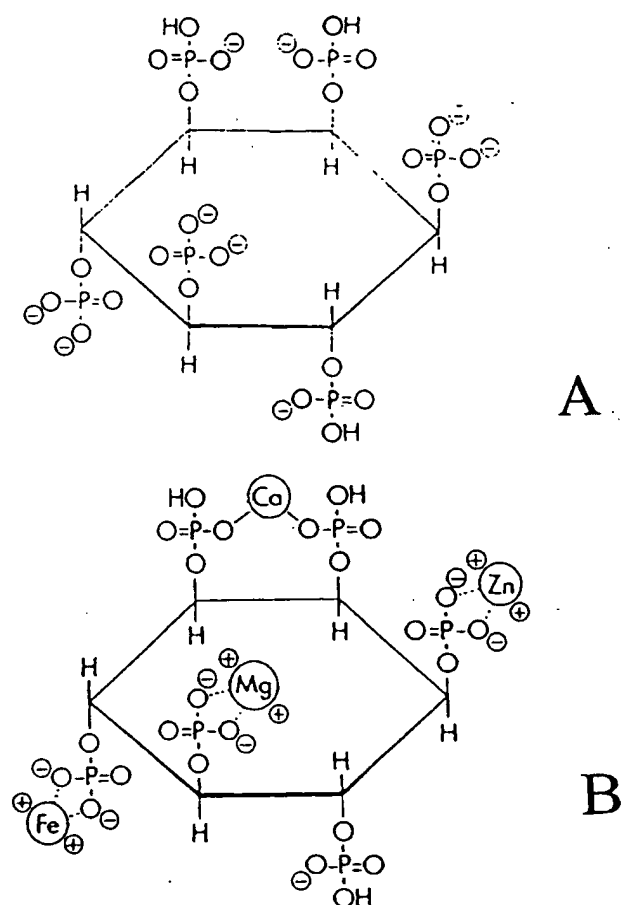


Fig. 1. Structures of phytic acid (A) and a phytic acid chelate (B) at neutral pH. Reproduced with permission, from Erdman Jr ref.15.

to 89%) values for soybean meal, cotton seed meal and rapeseed meal. However, these levels of calcium are too low for the optimum performance of birds.

The effect of the calcium:total phosphorus ratio on phytate phosphorus utilization has been reviewed by Wise.⁸⁹ A high calcium level or a calcium:total phosphorus ratio of 2:1 results in lowered digestion of phytate, owing to the formation of an insoluble calcium phytate in the intestine.⁹ Vandepopuliere *et al.*⁹⁰ found that, when diets contained 0.28% phosphorus entirely from plant sources, chicks fed a diet with a calcium:total phosphorus ratio of 1:1 performed better than those fed a diet with a 2:1 ratio. However, when the phosphorus level was increased to 0.72%, the widening of calcium:total phosphorus ratio did not appear detrimental. That chicks can utilize phosphorus from phytic acid, provided that calcium:total phosphorus ratio is in balance, was demonstrated by Harms *et al.*⁴⁹. They found that narrowing the calcium:total phosphorus ratio from 2:1 to 1:1 improved the availability of phosphorus from phytic acid to a greater extent than that from inorganic phosphate supplements.

5.2 Vitamin D₃

The importance of vitamin D₃ (1,25-dihydroxy-cholecalciferol), the active metabolite of vitamin D,⁹¹ in the normal absorption and metabolism of calcium and phosphorus is well documented.⁹² Early research clearly demonstrated that phytate utilization was depressed by feeding diets marginal or deficient in vitamin D₃, as discussed in a comprehensive review by Ewing.¹³ Several recent reports also indicate that the addition of vitamin D₃ markedly enhances the amount of phytate phosphorus retained by the chickens.^{8,44,45} In these studies, the phytate phosphorus utilization was increased from 31-50% to 68-87%. However, in other studies improvements due to vitamin D₃ supplementation were difficult to quantify because the magnitude of the response was generally small and influenced by other interrelationships.^{93,94}

The improved utilization of phytate phosphorus in response to vitamin D₃ supplementation may be attributed to one or more of the following three mechanisms: (1) increased synthesis or activity of 'intestinal phytase',^{63,95} (2) increased phytate hydrolysis^{45,96} via stimulation of calcium absorption, thus rendering the phytate more soluble and available for utilization, and (3) enhanced transport of phosphorus.^{97,98}

5.3 Age of birds

It is generally conceded that older birds hydrolyze phytate phosphorus to a greater extent than chicks,¹⁰³ the basis of this being that there is more of the phytase activity present in the gastrointestinal tract of older birds. The available literature suggest that with plant-derived ingredients about one-third of the total phosphorus is available to young chicks and perhaps up to one-half available to hens.

Edwards *et al.*¹⁰⁴ reported that the ability of poultry to utilize phytate phosphorus increases with age. Based on chromic oxide balance studies, they found that the 21-day-old broilers utilized the phytate phosphorus better than 14- and 7-day-old broilers; 59 vs 47 vs 35% retention. In this study, a significant effect of sex was also observed, with male broilers retaining more phytate phosphorus than females (39-62% vs 18-36%). Data of Ashton *et al.*¹⁰⁵ also indicated increased utilization of ³²P-labelled calcium phytate phosphorus by 6-week-old chicks compared to 4-week-old chicks; 36-49 vs 20%. However, Nelson,⁴¹ investigating hydrolysis of phytate phosphorus by 4- and 9-week-old broilers in balance experiments, observed only a slight increase in utilization by the older birds.

In retention studies, Maddaiah *et al.*¹⁰⁶ demonstrated that mature hens can use phytate

phosphorus more efficiently than the chicks. Several workers have shown that the phosphorus requirements of laying birds can be met entirely from plant-derived ingredients suggesting that a major portion of phytate phosphorus complex can be utilized by the hen to sustain egg production^{75,99,100,101} and egg shell quality.^{101,107} In contrast, Waldroup *et al.*¹⁰⁸ found that diets containing 0.34% phosphorus from plant sources were inadequate to support egg production or hatchability. The ingredients used in the dietary formulations probably contributed to this discrepancy. Whereas the earlier researchers employed diets containing wheat or wheat products, the diets used by Waldroup and co-workers were based on corn-soybean meal.

Interestingly, the ability of laying hens to utilize phytate phosphorus appears to decline with advancing age. Scheideler and Sell¹⁰⁹ reported that the retention of phytate phosphorus was quite high at 34 weeks of age, averaging 46.7%, but decreased to 9.1 and 16.5% at 50 and 72 weeks of age, respectively. The reasons for this decline with age are unclear.

5.4 Type of dietary ingredients

As discussed previously (section 4), phytate phosphorus utilization in poultry diets can be improved by incorporating plant-derived ingredients with known phytase activity. It has been demonstrated that phytate phosphorus in diets based on such ingredients, with no feedstuffs of animal origin, was well utilized by the young, growing chicken,^{72,74} pullets,⁷⁴ laying hens^{75,99,100,101} and turkey poults¹⁰² for production purposes and bone growth.

Differences in the solubility of phytate from different sources have been reported by Boland *et al.*²¹ They found that the phytate in soybean meal was more soluble than that in sesame meal. This would suggest differences in the extent of hydrolysis of phytates from different feedstuffs within the gastrointestinal tract, if one presumes that the soluble phytate is a better substrate for enzymic degradation.

5.5 Effects of fiber

There is some indication that certain fibrous sources have significant influence on phytate phosphorus utilization. In studies reported by Ballam *et al.*,⁴⁸ hydrolysis of phytate was reduced by cottonseed hulls but unaffected by wheat bran. At low dietary calcium levels, rice bran lowered phytate hydrolysis whereas alfalfa meal and cellulose caused improvements. The observed differences between fibrous feedstuffs may be related to the chemical variation within the fiber fraction. The dietary levels of fiber may also be expected to influence phytate

hydrolysis, but this aspect has not been investigated. Further research is needed to clearly establish any influence of fibrous feedstuffs on phytate phosphorus utilization.

5.6 Genotype of birds

Limited evidence indicate that there may be breed and strain differences in the utilization of phytate phosphorus. Edwards⁴³ reported the average retention of phytate phosphorus by Leghorn chickens to be greater than that by meat-type broilers (56 vs 37%). In this study, considerable differences were also noted between the three broiler strains that were tested in their ability to utilize the phytate phosphorus.

5.7 Processing of feed

Because of nutritional considerations, considerable attention has centred around the reduction of phytate levels in foods and current knowledge of processing effects on phytate degradation or phytate removal from plant foods has been reviewed.^{18,20} The processing methods commonly employed in human foods include germination, soaking, cooking, autoclaving, autolysis and fermentation. Of these, only autoclaving has some practical relevance in poultry nutrition.

In general, phytic acid is relatively heat-stable. Short-time cooking does not significantly affect the phytate content of legumes²⁰ and cereals.¹⁸ However, autoclaving at higher temperatures can cause significant destruction of phytic acid. Kratzer *et al.*¹¹⁰ reported that autoclaving of soyabean protein increased the availability of zinc to turkey poults and suggested that this increase was due to the destruction of phytic acid. This view was later confirmed by O'Dell¹¹¹ who found that most (88%) of the phytate in isolated soyabean protein was destroyed during autoclaving at 115°C for four hours. Summers *et al.*¹¹² found that autoclaving reduced the phytate: non-phytate phosphorus ratio in various feedstuffs, indicating some conversion of phytate phosphorus into the inorganic form. They also showed that commercial steam pelleting enhanced the utilization of phosphorus in mixed diets. However, when the diets contained high levels of wheat products, steam pelleting resulted in lowered phosphorus availability¹¹³ and this reduction was attributed to the destruction of endogenous phytase activity. Takemasa and Hijikuro¹¹⁴ also found that autoclaving of rice bran at 125°C for 3 to 5 hours decreased the phytate phosphorus content by 50% and increased the available phosphorus for chicks. Han¹¹⁵ reported that incubation of cottonseed meal in the presence of water at temperatures of 30 to 60°C resulted in significant reductions in the phytate content.

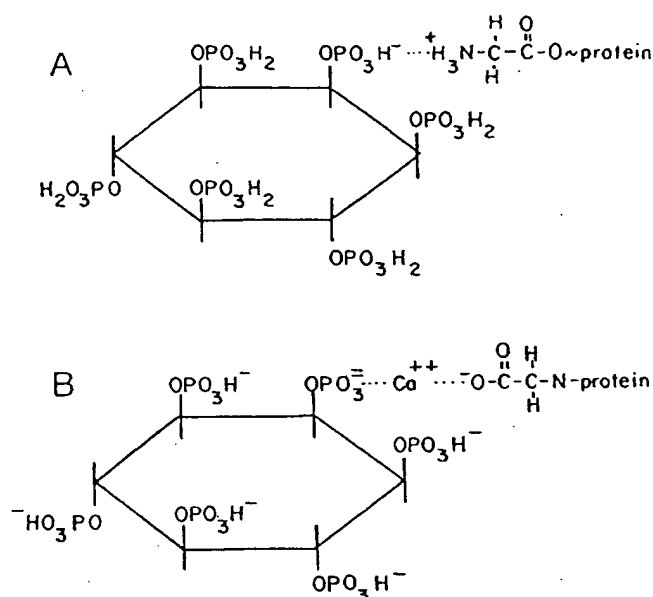


Fig.2. Phytic acid-protein complex at low pH (A) and neutral pH (B). Reproduced with permission, from Anderson, ref.151.

However, Lease¹¹⁶ showed that autoclaving of sesame meal reduced the phytate content by only 20%, despite a marked increase in zinc availability to chicks from autoclaved meal. de Boland *et al.*²¹ also reported phytate in sesame meal is resistant to autoclaving. Obviously, differences appear to exist between phytates from different feedstuffs in their response to heat treatment.

6 EFFECTS OF PHYTATE ON BIOAVAILABILITY OF OTHER NUTRIENTS

6.1 Minerals

The structure of phytic acid is suggestive of tremendous chelating potential (Figure 1). It is a strong acid and forms a wide variety of insoluble salts with di- and trivalent cations at neutral pH,^{32,117,118} potentially rendering these minerals unavailable for intestinal absorption. Whether or not a particular salt is formed depends on the pH, level of phytic acid and concentration of mineral as well as on the presence of secondary cations, of which calcium is probably the most important. The possible synergistic effect of two or more cations which may co-precipitate to increase the quantity of phytate-mineral complex is known.²⁰ This phenomenon has been demonstrated *in vitro* for zinc and calcium¹¹⁹ and for copper and calcium.³²

Vohra *et al.*,¹¹⁸ using titration curves of phytate as free acid in the presence of single cations, reported that

phytate formed complexes with minerals in the following descending order: Cu²⁺ Zn²⁺ Co²⁺ Mn²⁺ Fe⁺⁺⁺ Ca²⁺. In a similar study, Maddaiah *et al.*¹¹⁷ found that at physiological pH, zinc formed the most insoluble salt with phytic acid. The decreasing order of stability of salts was found to be Zn²⁺ Cu²⁺ Ni²⁺ Co²⁺ Mn²⁺ Ca²⁺. Although calcium has the lowest binding affinity, the greatest impact of phytate on mineral nutrition (other than phosphorus) is on calcium bioavailability.

It is now known that phytate lowers the bioavailability of several nutritionally important minerals, including calcium, magnesium, zinc and iron. Such interferences may lead to increased mineral requirements of animals. Copper, manganese, molybdenum and cobalt are other minerals that are believed to be affected. Several reviews have been published on this subject^{15,18,19,20,120,121} with primary emphasis on human nutrition.

Most published studies leading to the conclusion of the role of phytate on impairing the bioavailability of minerals have been conducted with humans and rats. Although some species differences with regard to mineral availability have been reported,¹²² it is likely that the findings have general application to poultry. In poultry nutrition, some attention has been given to the effect of phytate in increasing the requirements for calcium. Nelson *et al.*¹²³ found that the calcium requirement of White Leghorn chicks fed a purified diet containing no phytate was 0.5%. The requirement was increased to 0.95% on a practical diet containing 1.25% phytic acid. Similarly Farkvam *et al.*¹²⁴ found that increasing levels of phytate in the diet increases the calcium requirement to produce a given level of bone ash in broilers. Nelson¹²⁵ suggested that the dietary calcium requirements of poultry must be expressed in terms of available rather than total calcium. Thus, if diets were to contain ingredients high in phytate, more calcium would be required to offset the portion that was unavailable as insoluble calcium phytate. He recommended that the calcium needs of broilers be calculated as follows:

$$\% \text{ Dietary Ca} = 0.6 + (\% \text{ phytate phosphorus} \times 1.1)$$

However, interest in phytate continues to be more in relation to phosphorus availability, rather than calcium availability, owing to the expense of supplying phosphorus in the diet relative to the provision of calcium.

Zinc becomes a limiting mineral with high-phytate diets, since it forms a highly insoluble salt at pH 6.0,¹¹⁷ the approximate pH of the upper intestine where most of the absorption of minerals occurs. Zinc is also significantly affected by the phytate-calcium synergism. The effect of phytate on zinc availability of chicks has been demonstrated by several workers.^{110,116,126,127} O'Dell¹¹¹ reported that growth rate was lowered when

phytic acid was added to chick diets; this depression was overcome by the addition of supplemental zinc. The effect of calcium in accentuating the influence of phytate was demonstrated in a follow-up study by O'Dell *et al.*¹²⁸ Calcium aggravated zinc deficiency symptoms when it was added to diets in the presence of phytic acid. In the absence of phytate, excess calcium had no effect on zinc availability. Mills¹²⁹ also showed that zinc availability in phytate-rich diets is determined by the dietary calcium content owing to its synergetic effect on phytate-zinc antagonism. It was postulated that excess calcium increases the total cation concentration sufficiently to initiate a co-precipitation with zinc. The resultant zinc-calcium-phytate complex has been shown to be less soluble than zinc-phytate at pH 6.0.²⁰ Lease¹³⁰ showed that the zinc of sesame meal and safflower meal was present in an insoluble calcium-magnesium-zinc-phytate complex at intestinal pH and that it was poorly available to chicks.

The effects of phytate on the availability of minerals other than calcium and zinc have received only limited attention in poultry nutrition. McWard¹³¹ found that addition of 4% phytic acid - soyprotein complex to a balanced diet containing 75 ppm supplemental magnesium depressed growth and increased mortality of chicks. These negative effects were attributed to a decreased magnesium bioavailability. Guenter and Sell¹³² determined the availability of magnesium from different feedstuffs relative to magnesium sulfate, but their results showed that the magnesium availability of corn, wheat, barley and soybean meal were comparable to that of magnesium sulfate. Davis *et al.*¹³³ showed that diets containing isolated soyprotein with phytate lowered the availability of manganese and copper for chicks.

The extensive studies with phytate probably have led to an oversight of the effects of other plant components on mineral bioavailability. Plant-derived feeds that contain phytate also have fiber fractions which have a high affinity for minerals. The negative influence of different components of fiber, particularly of lignin and polysaccharides, on mineral utilization is well documented.^{28,134,135,136} However, Harland¹³⁷ concluded that phytate more than fiber components impairs mineral bioavailability. It was suggested that most of the effects of fiber on mineral binding are due to the presence of phytate. The data of Davies *et al.*¹³⁸ also indicate that phytate, rather than fiber, largely determines the bioavailability of zinc. Nevertheless, as suggested by Erdman,¹⁵ unless the phytate can be separated from the fiber components and evaluated independently, it may be difficult to attribute the adverse effects on mineral utilization to phytate alone.

Several reports indicate that the lower phosphates of inositol (inositol mono-, bis-, tris- and tetrakis-phosphates), which are formed during the step-wise dephosphorylation of phytate (inositol hexakis-phosphate), may have nutritionally less effects on mineral availability.^{139,140,141} It has been suggested that at least five of the six possible sites on inositol need to be phosphorylated in order to exert an inhibitory effect on the intestinal absorption of zinc and calcium. It would appear therefore, that the influence of phytate on mineral utilization may also vary depending on the degree of phytate degradation and the proportion of lower phosphates of inositol present in the gut.

6.2 Protein

The nutritional significance of phytate is further complicated by protein-mineral-phytate interactions^{142,143,144} and its inhibitory effects on proteolytic enzymes.¹⁴⁵ The association between phytate and protein begins in seeds during ripening when phytate accumulates primarily in the protein-rich aleurone layers of monocotyledonous seeds and in the protein bodies of dicotyledonous seeds.^{33,146}

The interaction between phytic acid and proteins is believed to be of an ionic type and is dependent on pH.^{14,147,148,149,150} At low pH, phytic acid forms electrostatic linkages with the basic arginine, lysine and histidine residues resulting in insoluble complexes (Figure 2). As the pH approaches the isoelectric point, the charge on the protein is neutralized and, the phytate is no longer bound and becomes soluble. In this soluble state, phytate complexes with protein because of the presence of divalent cations. These cations, usually calcium, magnesium or zinc, act as a bridge between negatively charged protein carboxyl groups and the phytate.¹⁵¹

In vitro studies have shown that phytate-protein complexes are insoluble and less subject to attack by proteolytic enzymes than the same protein alone.^{144,152} The reduced solubility of proteins as a result of such complexing can adversely affect certain functional properties of proteins which are dependent on their hydration and solubility. This aspect and implications in food systems have been reviewed.¹⁵³ Significance of phytate-protein complexes in nutrition, however, is still under scrutiny. Theoretically, phytate-protein interactions may influence the digestibility of protein. Whether this effect is significant depends on the character and configuration of the complex¹⁴³ which varies with protein sources; it appears that some proteins bind phytate while others do not. O'Dell and de Boland¹⁵⁴ suggested that the accessibility of binding groups is the key factor that determines phytate binding to the protein. Thus it is not surprising that conflicting results have been reported

from *in vivo* experiments with regard to phytate intake and protein utilization. While some have suggested that phytic acid does not affect protein digestibility,^{155,156,157} others have found improvements in protein utilization with decreasing levels of phytate.^{158,159} Satterlee and Abdul-Kadir¹⁶⁰ reported that the effect of phytate on protein utilization is influenced by the protein source. In their study, increasing levels of phytate had no effect on the protein utilization of soyprotein isolate, but decreased the utilization of high-protein wheat bran. Dietary phytate levels were correlated significantly with faecal nitrogen output, indicating that the lowered digestibility may have resulted from phytate-bound protein. However, it should be noted that changes in faecal nitrogen output may also be associated with other components of the diets, such as cell wall polysaccharides and lignins.¹⁶¹

Phytate is known to inhibit a number of digestive enzymes such as pepsin,¹⁶² α -amylase¹⁶³ and trypsin.^{145,164} This may be due to the non-specific nature of the phytate-protein interactions. Inhibition may also result from the chelation of calcium ions which are essential for the activity of trypsin and α -amylase, or possibly to an interaction with the substrates for these enzymes.¹⁶⁵ These negative influences may also be partly responsible for the effects of phytate on protein utilization. However, to what extent the inhibition of enzyme activity by phytate contributes to its overall anti-nutritional effect remains uncertain.

Anderson¹⁵¹ was of the opinion that the main nutritional effect of phytate-protein complex formation is the reduction in mineral availability. But several recent studies with supplemental dietary phytase provide indirect evidence that phytate-protein interactions can interfere with protein and amino acid digestibility.^{166,167,168,169,170} Rojas and Scott¹⁷¹ reported that the metabolizable energy contents of phytase-treated soybean meal and cottonseed meal were higher for chicks than those of untreated samples. They concluded that the increase in metabolizable energy was probably due to improved digestibility and availability of protein. Similar improvements in metabolizable energy contents were observed by Miles and Nelson¹⁷² for phytase-treated cottonseed meal and wheat bran but not for soybean meal. It was suggested that the beneficial effects may be dependent on the nutritional quality of the ingredient, being more effective on materials having poor protein quality and perhaps higher phytate content. Further research is warranted to understand the full implications of phytate on the protein quality of feedstuffs.

7 USE OF SUPPLEMENTAL PHYTASE AS A FEED ADDITIVE

7.1 Effects on phytate-phosphorus availability

Another aspect, not new but gaining greater prominence, is the use of microbial phytase as a mean of releasing the phosphorus from phytate, thereby reducing the need for inorganic phosphate supplements in feed formulations and the excess amounts of phosphorus excreted in manure; this has recently received considerable attention following the comprehensive studies reported by Simons *et al.*¹⁷³ Two decades ago, Nelson *et al.*¹⁷⁴ were the first to show that phytase produced by *Aspergillus ficuum* and other fungi could hydrolyze the phytate phosphorus in plant-derived ingredients. They incubated soybean meal with crude phytase extracts prior to feeding and found that chicks utilized the hydrolysed phytate phosphorus as efficiently as the phosphorus from inorganic sources. Phytate phosphorus in unincubated soybean meal was not utilized by chicks. In a subsequent study,¹⁷⁵ where the fungal phytase was added directly to a corn-soybean diet that was low in phosphorus, phytase addition resulted in increased bone ash indicating hydrolysis and utilization of phytate phosphorus. Similarly Rojas and Scott¹⁷¹ reported almost complete hydrolysis of phytate in cottonseed meals following treatment with a phytase from *Aspergillus ficuum*.

Although the low supply and high cost of microbial phytase¹⁷⁶ has limited its commercial exploitation in the past, the recombinant DNA technology currently available to synthesize the enzyme has generated new possibilities. This is evidenced by an upsurge of interest in the use of supplemental phytase to degrade phytate phosphorus and recent research results have been reviewed.^{10,177,178,179,180} Reported improvements in phosphorus availability are generally in the range of 20 to 45%, the amount of phytate phosphorus released being dependent on the level and source of added phytase and dietary phytate, dietary levels of non-phytate phosphorus, calcium and vitamin D₃, and calcium:phosphorus ratio.^{44,173,177,181,182,183,184,185,186,187,188} Other factors that are reported to influence phytate hydrolysis include temperature, moisture, pH and incubation time.^{173,189,190,191}

Unlike the plant phytases, microbial phytases are active over a wide range of pH, from 2.5 to 5.5.^{77,173} Thus they can be active within the proventriculus. Studies involving cannulated pigs have shown that over 85% of the hydrolysis of phytate by microbial phytase takes place in the stomach.¹⁹² A similar scenario probably exists in poultry with phytate hydrolysis mainly occurring within the proventriculus. This may explain, at least in part, the

effectiveness and consistency of microbial phytases compared to those of plant origin.

Obviously more research is needed to resolve the factors that may influence the efficacy of phytase enzyme in poultry diets and these factors include *inter alia* dietary levels of calcium, phosphorus and vitamin D. Future studies should also define the influence of calcium: non-phytate phosphorus ratio (rather than calcium:total phosphorus ratio) on phytase activity. The data of Edwards^{8,44} suggest that vitamin D and dietary phytase activity may function synergistically to enhance phytate phosphorus utilization. Available literature also indicate that high calcium levels may be detrimental to phytase activity.^{167,193,194} Efficacy of microbial phytases is probably different for various classes of poultry, due to differences in phosphorus and calcium requirements. However, very little is known about this aspect. Studies reported by Simons *et al.*¹⁷⁷ show that supplemental phytase is effective in layer diets despite their high calcium contents.

In the longer term, economics will determine the acceptability of phytase by the feed industry. This will depend not only on the magnitude of its efficacy, but also on the cost, product stability and ease of application.¹⁷⁸ Based on data from corn-soybean meal diets and using current US prices, Yi *et al.*¹⁹⁵ estimated that the cost of adding phytase enzyme to broiler diets is 1.3 times the cost of an equivalent amount of inorganic phosphorus; this cost however, did not include the cost of phosphorus disposal or the possible 'extra-phosphoric' benefits of the enzyme.¹⁹⁶ One can speculate that much attention will be centered in future studies on the effects of phytase on nutrients other than phosphorus and this, in turn, may increase the cost effectiveness of the enzyme.

7.2 Effects on availability of cations and protein

As previously discussed, the phytic acid has tremendous chelating potential and forms a variety of complexes with cations and proteins, rendering these nutrients biologically unavailable. Evidence is accumulating to indicate that the availability of calcium is improved by supplemental phytase.^{187,194,197,198,199,200} Studies on the influence of phytase on other phytate-bound minerals are limited. Pallauf *et al.*²⁰¹ found that phytase addition increased the apparent absorption of Mg, Zn, Cu and Fe by up to 13, 13, 7, and 9%, respectively, in pigs. In studies with chicks, Thiel and Weigand²⁰² reported improvements in zinc retention with phytase supplementation. In the studies of Roberson and Edwards,¹⁹⁹ added phytase had no effect on the zinc retention of chicks, but improved the retention when given along with vitamin D₃.

Theoretically, phytase supplementation must also be able to release the phytate-bound protein for utilization, but published data on this aspect is scanty. Officer and Batterham¹⁶⁸ observed improvements of 7-12% in the ileal digestibility of protein and essential amino acids in pigs with the use of supplemental phytase. Improvements in nitrogen digestibility^{167,169,170} and nitrogen retention²⁰³ have also been reported in several other studies. The possible effect of phytase on protein and amino acid utilization is of immense practical interest and needs to be confirmed in future studies with poultry.

8 CONCLUSIONS

Typical poultry diets contain 0.25 to 0.40% phytate phosphorus. The utilization of this phosphorus source remains a major problem area in the mineral nutrition research of poultry. Not only does this represent a potentially large source of phosphorus, should it become biologically available, this will also greatly reduce the amounts of phosphorus escaping digestion and absorption. There has been widespread concern in recent years about possible phosphorus pollution from animal manures; phosphorus is relatively immobile within the soil and excessive excretion in the manure would result in the accumulation of phosphorus over time, thus increasing the potential for contaminating water sources from runoff and erosion. Since phosphorus is the limiting nutrient for aquatic plant growth, the effect of increased content of phosphorus in water is eutrophication which decreases the available oxygen within the water thus posing a hazard to fresh water animal life. Effluent control is therefore a high priority in areas of intensive animal production and, in this context, phytase can become an important waste management tool.²⁰⁴

The availability of phytate phosphorus to poultry has been the subject of much controversy, and still there is no agreement on the extent to which it can provide the phosphorus requirements of poultry. However, there is now overwhelming evidence that phytate phosphorus is by no means completely undigested by poultry, especially older birds. The degree of utilization of phytate may vary widely depending on bird factors and various dietary conditions. Consequently, a single approach may not have universal application and different strategies will have to be considered to improve phytate phosphorus utilization in poultry diets. As stated by Taylor,²⁰⁵ it is the complexity surrounding the availability of phytate phosphorus that makes the subject fascinating.

It is generally assumed that about one-third of the phosphorus in feedstuffs of plant origin is non-phytate and is biologically available to poultry.²⁰⁶ Without doubt, this

is an oversimplification in view of the wide variability in the availability of phosphorus among plant feedstuffs.^{207,208} However, as noted by Nelson,⁹ this one-third availability of plant phosphorus could generally be applied to mixed ingredients although not to single ingredients. In this context, further research will be necessary to determine the amounts of phytate (and non-phytate) phosphorus in all available ingredients, especially new feed resources. Because conventional methods of phytate determination are time-consuming, only scattered and limited values for animal feedstuffs are available. The values currently in use have been based largely on few studies,^{27,48} improvement of this listing, using modern analytical techniques,¹⁷ will greatly aid in more precise balancing of phosphorus in poultry diets.

Phytase, either of microbial origin or endogenous to certain ingredients, must be present in poultry diets in order to hydrolyze significant amounts of phytate. Endogenous phytase in wheat has been shown to be effective in degrading phytate, but the degree of hydrolysis varies. The usefulness of commercial microbial phytases as a means of releasing the phosphorus from the phytate is highlighted in this review. Recent results have consistently demonstrated the efficacy of supplemental phytase in improving phytate-phosphorus availability and decreasing phosphorus excretion in manure.

In its native state, phytate is complexed with various cations and with proteins. In the foreseeable future, the effects of phytase supplementation on the availability of these biologically important nutrients will be studied and one can speculate exciting potential for wider application of phytase technology to enhance overall nutrient utilization in diets for monogastric animals. However, to be cost effective, more practical methods must be developed to improve the efficacy of the phytase enzyme. One area requiring immediate attention is the optimum dietary conditions to achieve maximum phytate degradation with supplemental phytase within the gastrointestinal tract. Success in this area will augur well for increased exploitation of plant-based diets. In these considerations, the relative significance of fiber components on nutrient availability should not be overlooked. It is imperative that the effects of phytate on the bioavailability of minerals and protein must be meaningfully evaluated in view of similar influence of fiber fractions on these nutrients.

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